Antimicrobial resistance in non-pathogenic *E. coli* isolated from slaughter pigs

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Abstract
An increasing antimicrobial resistance in bacteria of animal origin is recognised as a public health threat. Resistant pathogens directly affect infected host and can lead to therapeutic failures whereas commensal flora may serve as a reservoir and vector of resistance genes in a population. The incidence of resistant non-pathogenic bacteria may also indirectly indicate the intense of antimicrobial use in animal husbandry. A five-year resistance monitoring project covering an indicator E. coli was run at the National Veterinary Research Institute since autumn 2003. The study was designed to collect yearly up to 1000 samples from healthy bovine animals, pigs, broilers, turkeys and geese at slaughter. Standard isolation and identification procedures were applied for E. coli detection in bovine and swine rectal swabs and poultry caecum contents referred to the laboratory. Agar diffusion method according to CLSI recommendations was used for antimicrobial resistance testing of 1692 strains, including 676 swine isolates. Resistance ranged from 0.9% of tested strains (Cefuroxime) to 33.7% (Streptomycin). Certain year-to-year variations in the occurrence of antimicrobial resistance were noted. Resistance to at least 1 antimicrobial was found in 49.5% of strains isolated from pigs, compared to 16.6% of bovine and 89.1% of broiler isolates. Multiresistance was recorded in 6.4% of swine isolates in comparison with 2.7% and 39.7% found, respectively, in cattle and broilers. Eighty-two resistance profiles covering up to 9 out of 11 tested antimicrobials were observed in pigs isolates. The resistance in non-pathogenic E. coli depended on source of isolation. Swine isolates were more often resistant than bovine strains, but less frequently resistant than those obtained from poultry. It might be due to different intensity and prudence of antimicrobial use in animal husbandry.

Introduction

An increasing antimicrobial resistance in bacteria of animal origin is recognised as a public health threat. Resistant pathogens directly affect infected host and can lead to therapeutic failures. Commensal flora may serve as a reservoir and vector of resistance genes in a population. The incidence of resistant non-pathogenic bacteria may also indirectly indicate the intense of antimicrobial use in animal husbandry [3,6]. Those are the major rationale for monitoring and control of antimicrobial resistance. On the other hand knowledge on resistance and resistance patterns may be used as an effective empirical treatment during acute outbreaks of disease when an antimicrobial admission in needed as early as possible [2,5].
The problem is well known in bacterial pathogens although the occurrence of resistance varies between countries and regions [3,4]. A few and mostly point prevalence studies on the resistance among indicator bacteria originating from healthy animals are known and they support only limited information over a phenomenon within a longer time period.
The need for data on antimicrobial resistance was recognised in Poland in early 2000s. As a response a five-year resistance monitoring project was lunched at the National Veterinary Research Institute in autumn 2003. It covers E. coli, Salmonella, Staphylococcus, and Streptococcus. Nowadays the project provides an information on antimicrobial resistance in bacteria of animal origin crucial to the national program for antibiotics protection initiated in 2005 by the National Institute of Public Health and Ministry of Health.
The paper presents the resistance to a range of antimicrobials of non-pathogenic E. coli isolated from pigs, discusses the scope of the resistance, and compares the phenomenon in pigs and other animal species.

Material and methods

The study was designed to collect yearly up to 1000 samples from healthy animals sampled at slaughter in selected slaughterhouses located in 21 counties. Pigs were sampled by county veterinary officers at 8 slaughterhouses, whereas cattle, broilers, turkeys and geese at, respectively, 10, 5, 5, and 4 locations.

Standard isolation and identification procedures were applied for E. coli detection in samples referred to the laboratory. Rectal swabs (cattle and pigs) or a loopfull of caecum contents (poultry) were suspend in saline and streaked directly onto MacConkey agar. The plates were incubated in 37°C for 18±2h and a single lactose-fermenting colony was selected for biochemical confirmation. The number of isolates tested during the subsequent years were given in Table 1.

Agar diffusion method according to CLSI recommendation was used for ART. Mueller-Hinton agar and antimicrobial discs were manufactured by Oxoid. The antimicrobials and breakpoints used are listed in Table 2. Growth inhibition zone diameters were automatically read with OSIRIS (BioRad Laboratories) and the results were transferred to WHONet software for analysis.

Table 1. Number of isolates tested, by year and source of isolation

Year	pigs	cattle	broilers	turkey	Geese	total
2004	278	184	93	46	47	648
2005	306	181	59	24	36	606
2006	92	193	21	116	16	438
total	676	558	173	186	99	1692

Results

The E. coli isolation rate in the collected samples was high. Few samples yelled no lactose-positive culture growth on MacConkey plates and the only reduction in number of samples was due to motile Proteus spp cross-contamination.

The average resistance in pig isolates ranged from 0.9% to 33.7% in, respectively, cefuroxime and streptomycin (Table 2). In general, the percentage of resistant strains increased within 3-years period in the case of cefuroxime, chloramphenicol, gentamicin and TMP/Sulfamethoxazole, but only in the laser compound the change was significant ($P \leq 0.01$). The resistance to quinolones and sulfonamides ($P \leq 0.05$) peaked in 2005. In the same year the decrease in resistance for streptomycin and tetracycline ($P \leq 0.001$) was noted. A non-significant decline in the percentage of resistant strains within the study period was observed in the case of ampicillin and trimethoprim.

No matter the antimicrobial used, swine isolates were usually more resistant than bovine and less frequently resistant than poultry isolates.

Pig isolates showed 82 out of 191 noted resistance profiles and they were the most variable compared to other sources (data not shown). The percentage of fully susceptible isolates diminished from year to year and on average 49.5% showed any resistance (Table 3). The resistance profiles comprised up to 9 antimicrobials of all classes tested. The resistance to 5 and more antimicrobials, although higher than in cattle, was several times lower than in poultry isolates.

Table 2. Percentage of antimicrobial resistant E. coli, by source and year of isolation

Antimicrobial	*break*	*pigs*				*cattle*	*broilers*	*turkey*	*geese*
[code & concentration (μg)]	*point*	*2004*	*2005*	*2006*	*average 2004-2006*				
Ampicillin AMP (10)	*14-16*	10.4	9.2	8.7	9.6	9.3	61.3	41.4	33.3
Cefuroxime sodium CXM (30)	*15-17*	0.7	1.0	1.1	0.9	0.4	4.1	1.1	0.0
Chloramphenicol CHL (30)	*13-17*	1.8	4.2	4.3	3.3	0.5	11.3	10.8	5.0
Nalidixic acid NAL (30)	*14-18*	4.0	6.9	6.6	5.6	1.6	72.1	34.9	30.7
Enrofloxacin ENR (5)	*17-22*	0.4	2.6	2.2	1.6	0.4	33.5	11.8	3.1
Gentamicin GEN (10)	*13-14*	0.4	2.0	2.2	1.3	0.4	5.4	2.2	0.0
Streptomycin STR (10)	*12-14*	36.0	30.6	37.0	33.7	5.6	54.3	18.1	24.7
TMP/Sulfamethoxazole SXT (1.25/23.75)	*11-15*	6.9	11.8	18.7	10.7	4.3	26.6	13.3	20.8
Sulfonamides SSS (300U)	*13-16*	15.8	21.6	9.8	17.6	4.3	37.8	21.3	28.0
Trimethoprim TMP (5)	*11-15*	7.6	6.9	6.5	7.1	3.2	16.8	11.9	21.2
Tetracycline TCY (30)	*15-18*	34.6	20.1	44.0	29.2	8.7	53.8	44.6	22.2

Table 3. Percentage of susceptible, resistant and multiresistant E. coli strains, by source and year of isolation

Strains characteristics	*pigs*				*cattle*	*broilers*	*turkey*	*geese*
	2004	*2005*	*2006*	*average 2004-2006*				
susceptible	52.5	51.1	42.4	50.5	83.4	10.9	32.8	47.5
resistant to 1 antimicrobial	14.4	23.1	21.7	19.4	9.5	16.7	23.1	14.9
resistant to 2 antimicrobials	15.8	10.1	13.0	12.9	1.4	9.2	9.1	5.9
resistant to 3 antimicrobials	9.4	4.9	13.0	7.8	1.3	12.6	9.1	7.9
resistant to 4 antimicrobials	2.2	3.3	5.4	3.1	1.8	10.9	7.5	5.9
resistant to 5 and more antimicrobials	5.8	7.5	4.3	6.4	2.7	39.7	18.3	17.8

Discussion

Antimicrobial resistance surveillance programs run in some countries usually use diverse sampling frame, strain selection criteria, testing methodology. Therefore obtained results may not be comparable [2,3,7,8]. Being aware of the limitations we carefully discuss the general trends rather than the percentage of resistant strains observed in our study in comparison with those available from other EU countries.

Resistance to ampicillin, streptomycin, sulphonamides and tetracycline were the most common in E. coli both in Poland and other countries no matter the animal species considered [1,4,6]. Presented data show cephalosporins were the most active antimicrobial. Similar observations were found in Denmark [3] although the Dutch studies showed an increase of cefotaxime resistance in poultry isolates [6]. Some authors report the resistance levels of indicator E. coli tend to increase [3,6,7].

Certain year-to-year variations in the occurrence of antimicrobial resistance were noted during the study but the trends were mostly inconclusive. The resistance in pig isolates increased during the study period in the case of 8 antimicrobials tested although only TMP/Sulphametoxazole showed a significant change. An increasing trend in resistance was also observed in the stains originating from, respectively, cattle, turkey and geese (data not shown). No increase in resistance was observed in broiler isolates. Similar variations were described by others [3,6,8].

Host animal species and animal production system highly influenced the level of resistance. Poultry isolates showed higher resistance than those from swine. Similar tendency was observed in the Netherlands [6] whereas swine isolates were the most resistant in Sweden [8]. Cattle strains were usually the less resistant although compared to other antimicrobials the observed frequency of ampicillin resistance should be emphasized [1]. Penicillins usage in mastitis treatment might be the explanation. Quinolone resistance in broilers and other poultry species was higher than in to swine isolates [1,6]. Relatively infrequent gentamycin resistance served as an example of the lower selection pressure of the compound which is rarely used in animals [2,3]. Cattle are usually extensively reared whereas pigs and chicken are mostly housed under intensive conditions resulting in a higher proportion of resistance [1,6].

Antimicrobial resistance reflected regional differences in animal husbandry and antimicrobial usage [4]. For example, DANMAP [3] reported higher resistance in indicator E. coli isolated from imported broiler meat compared to the domestic ones. Prudent policy for antimicrobial use in Sweden results in a lower resistance. Therefore as much as 78% of indicator E. coli isolated from pigs were sensitive to all antimicrobials tested [8]. Similar conclusions can be drawn from Norwegian experiences [7]. A high antimicrobial consumption in Spain and the Netherlands is reflected by the higher incidence of resistance [2,6]. In our study nalidixic acid and enrofloxacin resistant E. coli indicate quinolone overuse in broilers whereas a short production period gives a limited opportunity for reduction of resistance once selection took place [6].

Conclusions

A harmonised antimicrobial monitoring and control programmes are needed throughout all Member States. Monitoring of antimicrobials consumption, restrictions and prudent antimicrobial use in food animals will reduce public health impact of drug resistant bacteria. It also concerns antimicrobial therapy in humans and the need for collaboration among veterinary, food hygiene and public health authorities.

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